IRIS LEAF BLOTCH

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IRIS LEAF BLOTCH

I. INTRODUCTION

The leaf blotch of Iris and several related genera has been known for many years. The disease is caused by the parasitic fungus Heterosporium gracile Sacc. It appears during the first warm spring days, but does not become very noticeable until summer and early fall. The disease manifests itself in oval or elliptical spots with brown borders, varying in size from one-fourth to one inch in maximal diameter. The tips of infected leaves die, and when infection is abundant the whole leaf dies prematurely.

Our knowledge of the disease dates back to 1881. In this year Saccardo (11)* published a description of the causal organism in Michelia. Subsequently, the fungus has been studied and reported upon by botanists of several of the European countries, but no work from a pathological viewpoint seems to have been done.

In 1893 Cooke (2) discovered the disease in England and briefly described it. Since this date it has become a very troublesome disease of Iris in some parts of that country. It was only in 1915, however, that any work of a pathological nature was done with the disease. In this year while connected with the Wisley laboratory, Mr. Ramsbottom studied the method

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* Reference is made by numbers to the bibliography.

of penetration and some physiological characters of the fungus.

In 1903-1904, Ritzema Bos (9) described the disease as attacking certain varieties of Narcissus in Holland. He conducted a series of spraying experiments from which he concluded that the disease may be practically controlled on Narcissus by the use of Bordeaux mixture.

In 1912, Güssow (6) reported the disease from Canada. It was reported as attacking certain varieties of Iris and Gladiolus in that country. Güssow recommended control measures but apparently made no studies of a pathological nature.

The first report of the disease in the United States dates back to 1888. In this year Mr. Ellis reported it from Newfield, New Jersey, on Hemerocallis fulva. Mr. Elam Bartholomew collected specimens of it at Stocton, Kansas in 1908. Mr. Baker collected it at Toledo, Kansas, the same year,* and specimens of it were collected by several members of the botany department of the University of Wisconsin at Madison, Wisconsin in 1913. Since this date it has become more or less troublesome in some of the gardens in Madison, Wisconsin, and has excited inquiry from some of the growers. Apparently, however, no research work of a pathological nature has been done with the disease at any point in the United States.

Thus, while iris leaf blotch has been known for many

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* Exsiccatae, Ellis and Everhardt, Fungi Columbiana, 2189, 1828, 2732.

years by botanists, it has received very little attention from pathologists, and the status of knowledge concerning the disease is somewhat fragmentary and incomplete. Only one attempt at a study of the malady from a pathological viewpoint seems to have been made, previous work having been confined to a description of the causal organism and the disease which it induces. Consequently, the detailed life history of the causal organism in relation to pathogenesis and control measures has remained incomplete.

This status of affairs seemed to warrant a further inquiry into the trouble, and it has been the subject of the writer's investigation for the past year in the University of Wisconsin.

Special attention has been given to the method of infection and the seasonal development of the fungus in relation to the production of the disease. Some control measures were planned and begun, but have not been carried far enough to warrant any definite conclusions in this paper.

II. THE DISEASE

Geographical distribution and economic importance.

Iris leaf blotch seems to be almost universal in distribution. Cooke (3) reports it from Britain, France, Italy, Germany, New Zealand, and Cape of Good Hope. Lindau (7) reports it from Holland, Greece, Denmark, and Tyrol. It has been more recently reported from Canada.

Nothing is mentioned of the importance of the disease

in any of these countries except Holland, England, and Canada. Cooke (4) writes: The iris leaf blotch is one of the most persistent and troublesome of iris diseases. It appears at some seasons with astonishing vigor upon the leaves of Iris germanica and other species.

Mr. Ramsbottom (8) writes:

With us the disease is noticed particularly in late summer and early autumn, during August and September, but it is often present on the leaves in early spring. ..... The affected plants may flower for a few years but eventually become weak and die.

Mr. Güssow (6) writes:

The disease usually does not develop until comparatively late in the season, and the damage done to the plants is not so serious as would otherwise be the case.

In order to determine to some extent the distribution and economic importance of the disease in the United States a short questionaire was sent to the plant pathologists of the following states: Illinois, Iowa, Michigan, Minnesota, Missouri, Ohio, Indiana, and New York. It was reported from Ohio and New York, as being widespread but of little economic importance.

The disease occurs very abundantly in some of the gardens in Madison, Wisconsin, and detracts considerably from the appearance of the plants during the summer and fall. The affected leaves die prematurely, which naturally reduces the amount of food to be stored in the root stocks. This of course weakens

the plants developing the following spring and, if the process is repeated indefinitely, will ultimately kill the plants.

Hosts. On account of great variability and wide distribution there are many synonomous terms for the species and horticultural varieties of the genus Iris. In a general way the members of the genus are classified into six groups, viz. (1) German, (2) Japanese, (3) Tall Apogon, (4) Dwarf, (5) Oncocyclus, and (6) Bulbous. Although there are no very great differences among the groups of the genus, it is interesting to note that the fungus H. gracile has never been known to become parasitic upon any except members of the German group. This group is said to prefer a lime soil, and is usually attacked most severely when growing on soils deficient in lime(?).

In addition to the group of German irises, the fungus has been reported as being parasitic upon several other genera of the Iridaceae family and also upon some genera of the Liliaceae family. Cooke (3) states that the fungus has even extended to Lychnis, a genus of the Caryophyllaceae family. The other genera upon which the fungus has been reported are, Friesia, Antholyza, Hemerocallis, Gladiolus, and Gemmingia. Ritzema Bos (9) reports it upon the following horticultural varieties of Narcissus, Bicolor Horsfieldii, Lorifolius Emporor, Bicolor Empress, Golden Spur, Henry Irving, Sulphur Trumpet, Sir Watkin, Orange Phoenix, Incomparable, Trumpet Major, and Trumpet Maximus. In Wisconsin it has not been found upon any other than iris plants.

It is difficult to conceive of a fungus attacking different genera of a family and even of different families, and yet fall short of parasitism on species within a genus. So far the fungus has been studied only by botanists and mycologists whose work has been limited to a description of the morphological characters of conidiophores and conidia. It is very probable that further investigation will show distinct species of the fungus on the different host genera.

Symptoms. Cooke (3) describes the disease on iris as follows:

The disease occurs as elliptical or oblong spots, about 1/4 to 1 inch in length, darker at the edge. At length the spots become confluent until the whole surface of the leaf is brown and dead, while the original spots remain of a paler color than the surrounding tissue. Often there is no external evidence of the presence of a fungus in the spring, but there is a plentiful fungus mycelium in the host tissue. Ultimately the tufts of conidiophores occur in the center of the spots.

Mr. Güssow (6) writes:

The iris leaf blotch occurs as small, round, elliptical, or occasionally more elongated spots of a paler brown color, surrounded by a conspicuous dark brown border, 1/2 inch in diameter. Center paler with age and shows numerous minute black spots, which are tufts of spore bearing hyphae. The leaf tissue adjacent to the spots becomes yellowed, and when these are numerous the whole leaf withers up prematurely.

Ritzema Bos (9) describes the disease on Narcissus as follows:

Kurze Zeit nach dem Blühen der Narzissen - jedenfalls niemals vor Anfang Mai - färben sich die bis dahin gesunden, grünen Blätter gelb, bewohnlich zunächst an den Ränden; bald verbreitet sich die gelbe Färbe über das ganze Blatt, welches ziemlich schnell dürr wird. Inzwischen bedeckt sich die Ober fläche der verdorten Blätter, resp. Blatteile, mit einem schwärzlichen Anflug.

From this description we see that the Narcissus is affected in a somewhat different manner than the iris.

In the earliest stages the infection on the iris occurs as very small, brown dots with a watersoaked halo around them (plate 5). These areas are similar on both surfaces of the leaf. As the spots grow larger this watersoaked halo turns yellow. The spots enlarge very slowly on young leaves in the spring, requiring more than a month to attain a diameter of 1/4 inch, and produce conidia. Plate 6 shows a mature spot enlarged about five diameters.

III. THE CAUSAL ORGANISM

Taxonomy

Saccardo (11) (1881) first described the fungus causing iris leaf blotch naming it Heterosporium echinulatum (B. & Br.) Cooke. This specific name, however, had previously (1870) been given by Berkeley and Broome to the fungus attacking car-

nations. Saccardo (1886), therefore, changed the name to Heterosporium gracile (Wallr.) and published an emended description in his Sylloge (10). Subsequently (8), the specimens which De Thumen had in his exsiccatae labeled Helminthosporium gracile Wallr. were found to be a Brachysporium instead of a Heterosporium as Saccardo had assumed. Therefore, the specific name gracile as applied to Heterosporium has no connection with Wallroth's description and the binomial becomes Heterosporium gracile Sacc. (8).

Morphology

Mycelium. The morphological characters of the mycelium vary slightly with the conditions. The very young hyphae are delicate, hyaline, branched and distantly septate. On most media used this type of hyphae forms a dome-shaped aerial colony. After a few days the hyphae in contact with the substratum increase in diameter, the cells grow thicker and become olivaceous to dark brown in color and form a leathery stroma on the surface of the media. Such hyphae are also found in the dead tissues of the spots on green leaves. During the development of the spots small, dark colored stromata develop in the substromatal chambers from which conidiophores arise.

The mycelium which passes the winter in the tissues of dead infected leaves gives rise in early spring to "perithecioid" (J. J. Davis) stromata. During the incipient stages of development these bodies are filled with small oil globules, but as they grow older the oil globules disappear, leaving them

hollow in the center. On the approach of warm weather tufts of conidiophores develop on the apex of these perithecioid stromata (plate 7) and bear conidia. Many sections were made of the stromata at all stages of development but nothing resembling asci was ever found. It seems that the stromata start out to produce perithecia and asci, but find conditions more favorable for the production of conidia, which they do instead. This leaves them as perithecioid stromata.

Somewhat similar structures are produced in pure culture on melilotus stems and cooked iris leaves (plate 8). The incipient development in this case is just beneath the cuticle, The cuticle is soon ruptured and further development takes place above the surface. These vary in size from 175 to 250 microns in diameter. They also contain oil globules while young and become hollow after about 40 days. Many cultures bearing these perithecioid bodies were kept at various temperatures from one to three months in an effort to stimulate the production of asci, but without avail.

Conidiophores. The conidiophores arise from stromata in the substomatal chambers in spots on green leaves and push out through the stomata. Lindau (7) describes the conidiophores from leaf lesions as follows: Konidienträger septiert, nach oben hin knotig, olivengrün, 70 microns lang, 10-11 microns dick.

The description according to Saccardo (10) is as follows: Hyphis in aëolis foliorum arescentibus, fusco-cinctis enascentibus, crassis, septulatis, 70=10-11, sursum nodulosis subolivaceis.

These descriptions practically agree with the conidiophores taken from lesions on green leaves and studied by the writer. The ones developing in the spring on the perithecioid stromata, however, are much longer. The dimensions of these range from 130 x 11 microns to 175 x 13.6 microns, the average being 156 x 12 microns (Plate III.)

The conidiophores produced in culture are much longer and more flexuous (plate II) than the ones produced in nature. The conidia are borne singly at the apex and as the spore approaches maturity it is pushed aside and the conidiophore continues, producing more spores in like manner. This method of growth gives the conidiophore in culture a zig zag appearance.

Conidia. The conidia develop acrogenously and singly (plate II). In the incipient stages they appear as small, conical, light olivaceous projections at the apices of the conidiophores. They develop very rapidly (within 24 hours in moist chamber) and produce the septae only after they have attained their maximum size. The echinulations develop early. The spores are attached to the conidiophores by short papillae (plates II and 7) from which they are readily displaced at maturity, leaving the papillae adhering to the conidiophore.

Saccardo (10) describes the conidia as follows:

Conidiis tereti - oblongis, 40-60 = 18-20, saepius, 2-3 - septatis atque constrictis, distincte muriculatis, pallide olivaceis.

Lindau (7) describes them as being langlich, cylin-

drisch, dautlich stachig, blöss olivengrün, meist mit 2-3 Scheidenwänden und eingeschnürt, 40-60μ lang, 18-20μ dick.

These descriptions compare very favorably with the spores taken from lesions on green leaves, except that no muriform spores were found. Conidia produced in pure culture are lighter colored, more slender, and apparently thinner walled, than the ones produced in the field. Conidia from both the field and pure culture were measured and the results are given in table I.

Table I

Comparison of spores from field with those from pure culture

Source of conidia	No. measured	Av. measurements (micron)		Max. meas. (microns)		Min. meas. (microns)	
		Length	Breadth	Length	Breadth	Length	Breadth
Lesions on green lvs.	100	50.8	16.8	61	18	43.7	13.7
Culture on melilotus stems	100	63.3	13.9	86.	15	34.	11.

Physiology

Cultural characters

Cultures from single spore isolations of Heterosporium gracile were grown on six different kinds of media. The fungus grew slowly on all media, and showed only minor variations upon all substrata except the melilotus stems and cooked

iris leaves. The cultural characters on the different media are outlined as follows.

Prune agar slants. In diffuse light in the laboratory, at 18° to 22°C. the conidium puts out one to three germ tubes from most cells, each of which branches within 8 to 10 hours. At the end of two days, a small white colony is formed on the surface of the medium. The cells of the hyphae are thin walled, hyaline and long. Within five days the colony becomes dome-shaped and about six millimeters in diameter. After 9 or 10 days the center of the colony is greenish gray to olive-green due to the presence of conidiophores and conidia. The cell walls of the hyphae in contact with the substratum soon begin to turn brown and grow thicker and by the end of fourteen days have formed a thin, leathery, dark brown stroma. At the end of about thirty days this covers the whole surface of the slant, and all the surface growth is olive green in color.

Potato agar slants. The early growth closely parallels that on prune agar, except that sporulation and stromateoid development begins about two days earlier. The stroma begins to warp at the end of twelve to fourteen days, giving the colony a characteristic contoured appearance. This warping of the stroma is accompanied by a cracking of the agar underneath. The hyphae then grow down into the cracks to some extent. At the end of one month the surface is dark green, and deeply contoured.

Corn meal agar slants. Growth is slower and the aerial hyphae less abundant than on either prune or potato agar. The

colony never develops the dome shape. Conidia are sparsely produced. A very dark stroma is produced but it seldom covers the surface of the slant before the agar dries out.

Iris decoction agar slants. Early development closely parallels that on potato agar, except after a few days it develops a light brown or salmon color. The aerial growth does not settle down subsequently as it does on the other media. The stroma becomes very dense and after about 20 days a few perithecioid stromata develop.

Nutrient agar slants. The early development is very slow and scant. The colony is distinctly spreading with little aerial growth. A dome-shaped growth of delicate hyphae penetrates the substratum almost to the wall of the tube. Few conidia are produced, and the dark stroma never develops. The agar usually dries out before the colony covers the surface of the slant.

Melilotus stems. When inoculated with mycelium, a white colony develops at the point of inoculation and spreads over the surface rather slowly, covering a distance of about three inches in two weeks. Conidia were produced at the end of two days, and as the conidiophores became more numerous the colony presented the characteristic olive-green color. After eight or ten days the white aerial growth began to disappear from the center. At the end of twelve days the perithecioid stromata begin to develop and become very numerous within twenty days after inoculation.

Cooked iris leaves. The early development closely par-

allels that on melilotus stems, except that it is more vigorous. The perithecioid stromata begin to develop just beneath the cuticle and soon break through, reaching their maximal size in about one week.

Relation of temperature to mycelial growth

In order to determine the temperature best suited for mycelial growth, three series of prune agar cultures were run in duplicate at stated temperatures, ranging from 4° to 35°C. It was hoped to compare the temperature thus determined with that at which infection takes place. A uniform quantity of agar was used in each plate. The plates were inoculated in the center with mycelium transferred from a 10-12-day-old prune agar culture of Heterosporium gracile. The plates were poured and inoculated under a transfer hood and placed at the different temperatures immediately after inoculation. The diameters of the colonies were measured at intervals of five days, and the average results of the three series at the end of ten and twenty days respectively are shown graphically in plate 10.

Resistance to cold

Conidia. The conidia of Heterosporium gracile are capable of resisting a very low temperature for a short period of time. Spores which were taken from the field after the host plants had been covered with snow for several days and the temperature had dropped to about 5° Fahrenheit, germinated very

readily in drops of distilled water on glass slides. The few spores which could be found one week later were not viable.

After making these observations it was thought worth while to subject conidia in pure culture to low temperatures in order to determine, if possible, how many degrees of freezing they could withstand. For this purpose two tubes each of 10 to 14-day-old prune agar, potato agar, and cooked iris leaf cultures of the fungus were placed on the ledge of a north window and covered with a glass jar. The night after the tubes were exposed the temperature dropped to -10° Fahrenheit. Spores taken from each of the cultures the following morning and placed in drops of sterile water on glass slides germinated within three hours. Germination tests were made subsequently in a similar manner at intervals of three or four days and after three weeks had elapsed none of the spores were viable and many of them had disintegrated. This disintegration is not due to the effects of cold, as will be shown subsequently.

Mycelium. The mycelium is capable of living throughout the winter in pure culture kept under outside conditions. The cultures used for this test were the same ones used for the above experiment. The lowest temperature recorded during the experiment was -20° Fahrenheit. Transfers were made to culture media at intervals of about two weeks, and in every case vigorous growth developed. The cultures were left outside until late spring and numerous conidia were produced. Production of conidia under these conditions occurred about two weeks earlier than

on dead leaves in the field. This is possibly due to the differences in moisture content.

Spore germination studies

Many germination tests were made with conidia from pure culture and from the field material. The spores were placed in drops of sterile distilled water and prune decoction on glass slides, and in water agar and prune agar in petri dishes. The cultures were then incubated under various conditions. For germination tests alone the open drop cultures on glass slides were most convenient and satisfactory.

In water. In diffuse light or in darkness, at 20° to 26°C. the conidia from pure culture begin to germinate after about two hours and thirty minutes. Incipient germination is manifested by hyaline protuberances of the spore walls (plate). All cells of some spores germinate at about the same time, the middle cells of others more slowly than the end cells, and some cells of certain spores never germinate at all. Some cells produce as many as three tubes (plate 1). These protuberances rapidly develop into hyaline, septate germ tubes 4-7 microns in diameter. At the end of 8 or 10 hours the tubes had attained a length of the spore, or more, and were beginning to branch. No attempt was made subsequently to keep track of an individual tube. At 30°C. the spores required about 11 hours for germination and the middle cells usually failed to germinate. The germ tubes grew very slowly, and were densely vacuolate (plate IA). No branches or septae had developed at the end of two days.

Conidia taken from the field varied considerably in the time required for germination at any given temperature. In some cases they germinated within 45 minutes while again the time was about the same as that for spores taken from pure culture. This variation was possibly because germination had already begun when taken from the field.

In prune decoction. In drops of about one per cent prune decoction, the time required for germination at all temperatures was about the same as in sterile water, the effects of the decoction being manifested in the rapidity and vigor of development of the germ tubes, The tubes began to branch about one hour sooner, and increased in length more rapidly than in water.

In prune and water agar. In these media, the time required for germination was practically the same as in distilled water. Early development of the germ tubes was not quite so rapid and vigorous as in the prune decoction, but branching seemed to be more profuse after once started. Colonies developing from single spores were examined daily, and after 8 to 10 days conidia were found present.

Relation of temperature to spore germination

In order to determine the temperature best suited for the germination of conidia two series of tests were made at constant temperatures ranging from 4° to 35°C. The conidia were taken from a 14-day-old culture on melilotus stems. The spores

were suspended in sterile distilled water so that each drop contained several spores. A drop of uniform size was placed on flamed slides and run in duplicate at each stated temperature. The slides were rested on small pieces of wood in the bottom of petri dishes containing a piece of moist filter paper on the bottom. The dishes were immediately placed at the constant temperatures, and removed at intervals of 20 minutes for examination, the least possible time being used for each observation. The ones at 4°C. required several hours for germination and were not examined during the night. The average results obtained are shown in table II.

Table II

Relation of temperature to spore germination

Temp. degrees C.	Time required for germination
4	21 hrs. 15 min.
8	6 "
13	4 "
17	3 "
22	2 " 30 min.
26	2 " 45 "
30	11 "
35	No germination

These results show that the temperature requiring the shortest time for germination is also the optimal temperature for mycelial growth (plate X).

Pathogenicity

Isolation of the organism

Two methods were used for isolating the causal organism from the diseased leaves, viz., (1) leaf fragment method, and (2) spore dilution method. Small pieces of leaf were cut from the borders of spots on green leaves, dipped in 95 per cent alcohol to remove the air and then immersed in 1-1000 mercuric bichloride solution for one minute. They were then rinsed through three sterile water blanks for five minutes and plated on prune and iris decoction agars. Apparently only one kind of growth developed after from 5 to 7 days. Transfers were made to agar slants and carefully examined later to make sure that only one organism was present. For the dilution method spores were taken from the spots on green leaves and suspended in sterile distilled water so that each drop of the suspension contained one or more spores. One drop of such suspension was added to a tube of melted water agar cooled down to 40°C., rolled, and poured into a plate. After the agar congealed the platewas inverted on the stage of a microscope and the spores located with the low power. Their position was marked with ink so that subsequent development could be readily observed. If no other organism developed near each single spore colony within two or three days a small disc of agar containing the spore was cut out with a flamed flattened platinum wire loop, lifted out and transferred to agar slants. Some trouble was experienced with

bacterial growth at first, but after the technique was developed the organism could be readily obtained in pure culture.

Inoculation experiments

The inoculation experiments with Heterosporium gracile were conducted along two lines, viz., (1) inoculation of the varieties of German iris attacked by the parasite, and (2) cross inoculation on Siberica iris, Narcissus Pheasant and Emperor, and a species of Hemerocallis. All of the plants inoculated were grown in the greenhouse where the temperature ran fairly constant at about 22°C. Part of the plants were grown in deep soil, the others in six-inch pots.

The plants used for the first experiments were taken from the field in November. Some of these plants were planted in deep soil in the greenhouse, the others in six-inch pots and placed outside the greenhouse until January 15. They were then removed to a cellar and allowed to thaw out slowly. When young leaves began to appear the pots were taken out of the cellar and placed under a greenhouse bench where they were exposed to the morning sun. The plants were watered too heavily at first and some of them became affected with soft rot and died. After the water supply was reduced the unaffected plants made vigorous growth. Other plants were taken from the garden while the ground was yet frozen and, after being allowed to thaw out in the cellar, were planted in six-inch pots and placed under the bench where the first ones were kept. These grew uniformly

and, so far as the writer could determine, normally.

Inoculation

Three methods of inoculation were used, viz., (1) inserting mycelium into the leaf tissue with a flamed needle, (2) sowing the spores on marked areas of the leaf surface in drops of sterile distilled water, and (3) spraying the plants with a sterile distilled water suspension of spores. Previous to inoculating, the bloom was removed from some of the plants by lightly rubbing the leaves with a piece of clean cheese cloth. Immediately after inoculation the plants were placed in moist chambers lined with moist toweling paper. When signs of the disease appeared the plants were taken out of the moist chamber and placed on a greenhouse bench. The range of temperature during one series of experiments is shown by the thermograph record in plate 9. One to two plants were used for control in each case, depending on the number inoculated.

Source of inoculum. The mycelium used for inoculation was taken from 14-day-old prune agar cultures of a single spore strain of Heterosporium gracile. The conidia were taken from 14 to 16-day-old melilotus stem cultures made from a single spore strain. "Sporiferous" suspensions were obtained by pouring the sterile water into tubes containing the culture and slightly agitating the tubes. In this way a suspension was made practically free of mycelial fragments.

Germination tests were made on flamed glass slides

with drops of the spore suspension used for inoculation. In each case the spores were found to be viable, and germinated in the usual time for that temperature. The results of the inoculation experiments are shown in table III.

Table III

Results of inoculation experiments

Kind of plant	Source of inoculum	Period of incubation	No. inoculated	No. infected	Percentage infection
German iris	Mycel.	2 days	3	3	100
" "	"	" "	4 (12 lvs.)	4 (12 lvs.)	100
" " var. honorable	"	" "	3 (6 ")	3 (6 ")	100
Ditto	Spores	5 "	2	2	100
Hemerocallis sp.	Mycel.	----	2	0	0
" "	Spores	----	2	0	0
Siberica iris	"	----	2	0	0
" "	Mycel.	----	3 (12 lvs.)	0	0
Narcissus emperor	"	----	2 (6 ")	0	0
" "	Spores	----	4	0	0
Hemerocallis sp.	Mycel.	----	3 (12 lvs.)	0	0
German iris	Spores	8 days	3	3	100
" "	"	5 "	6	6	100
" "	"	5 "	2	2	100

The results recorded in table III show that the varieties of German iris used are very easily infected with both mycelium and spores of Heterosporium gracile. Where mycelium was the source of inoculum, infection took place very readily, and after six days the spots were about one-half inch in diameter and bearing conidia. The lesions produced by spore infection on the other hand develop very slowly, attaining a diameter of only one or two millimeters in 30 days in the greenhouse. One plant was placed out doors just after infection occurred, and at the end of 35 days the lesions were about one-half inch in diameter and conidiophores were present. Just why the difference in the two cases is not clear to the writer, as the greenhouse temperature was about the optimum for mycelial development, while that outside ran down almost to the freezing point two or three times during the test. It might be that the plant outside was less vigorous than the ones inside. This point needs further investigation.

In no case were any of the youngest central leaves infected with spores, even when the older outer leaves were abundantly infected. No investigation was made to determine the reason for such a phenomenon, but it may be that the stomata of the young leaves are smaller or the fungus enters these leaves and falls short of parasitism. Lack of time prohibited investigation of this point.

The leaves from which the bloom was removed always received more abundant infection than the ones not so treated.

This was possibly due entirely to the fact that the inoculum spreaded very uniformly over the surface where the bloom had been removed, while it formed in drops on the untreated ones and would often run off.

Reisolation of the fungus

The organism was reisolated from several of the young lesions developing from artificial inoculation by the leaf fragment method, and its identity confirmed.

Relation of moisture to infection

In order to obtain some idea as to the amount of moisture necessary for infection, two series of experiments, run at different times, were conducted with Iris honorable. Plants were sprayed with a sterile water spore suspension just as in the other inoculation experiments and then subjected to different degrees of humidity. After inoculation, two plants in each series were placed on a bench in the greenhouse, two in a moist chamber for 14 hours, and two in a moist chamber for 5 days. The plants on the bench dried off over night and no infection ever developed. The ones kept in a moist chamber for 14 hours developed slight infection in each case, while the ones left in the moist chamber 5 days developed abundant infection. This experiment was conducted with only a few plants and will not justify any definite conclusions, but it shows that a certain amount of moisture is necessary for abundant infection.

Rhizome inoculation

The fungus has never been reported as attacking the underground portions of iris plants, but the writer thought it worth while to determine if the fungus possessed the ability to attack these parts.

Sound rhizomes of German iris were immersed in 1-1000 mercuric bichloride solution for five minutes and then rinsed in sterile water. Slices about 1/4 inch thick were cut with flamed scalpel under a hood. Three slices were placed in each of three petri dishes containing moist filter paper in the bottom. Two of the slices were inoculated with mycelium of Heterosporium gracile and the third was used as a control. At the end of eight days a dome-shaped colony about one-half inch in diameter had developed and was sunken below the general surface. It seemingly used up the cell contents which caused a shrinkage of tissue. The tissue for some distance out from this sunken area was brown and soft. Six days later practically all of the tissue was brown, shrunken, and spongy. The controls were slightly dried out but otherwise unchanged. The experiment was repeated a week later with similar results.

The fungus can also attack the rhizomes through the unbroken epidermis. Uninjured ~~root stocks~~ rhizomes which were sterilized as previously mentioned were inoculated by placing blocks of agar containing the fungus mycelium on the surface. They were then placed in a moist chamber and kept at laboratory temperature. After 24 days a brown area was present around the

point of inoculation, and when cut open the whole interior parts were found to be brown and spongy. The checks remained sound throughout the experiment.

IV. LIFE HISTORY OF THE CAUSAL ORGANISM IN RELATION TO PATHOGENESIS

Seasonal development of the disease

Primary infection of the season takes place 5 to 10 days after the first warm spring rain. This was during the first week of May in Wisconsin. Secondary infection occurs at any time throughout the summer when moisture conditions are favorable. The young lesions develop very slowly at first, reaching a maximal diameter of about 5 or 6 millimeters by May 25. At this time some of the lesions are producing conidia. Conidia are not very abundant, however, until about the middle of June. By this time the spots are from one-fourth to one-half inch in diameter (specimens taken by Mr. Carsner last June). The infection becomes more abundant throughout the summer and by the first of October many of the leaves are dead or partially dead.

Sources of infection

The sources of primary infection are conidia from dead infected leaves and conidia from lesions on leaves which have remained green over winter. Careful field observations were made from the time the snow melted until the first macros-

copic signs of infection appeared, and the atmospheric conditions noted. No spores of Heterosporium gracile were found until during the three rainy days of the last week of April. At this time abundant conidia developed both on the dead infected leaves and on the few lesions occurring on the old green leaves. Six to eight days later abundant young infection was found. It seems very probable that inoculation occurred during the rainy days as these new conidia were the first evident source of infection and the period of incubation corresponds very favorably with that on artificially inoculated plants in the greenhouse.

Mode of entry of the organism and the subsequent development in host tissue

Infection takes place through the stomata, and the hyphae subsequently become inter- and intra-cellular in the host tissue.

Numerous sections of both naturally and artificially infected leaves have been made, at intervals varying from one day to one month after inoculation. Study has also been made of the epidermis, which was removed and stained without sectioning. For this study leaves were taken from inoculated plants in the greenhouse. Also healthy green leaves were placed in a moist chamber and inoculated by sowing conidia on the surface in drops of distilled sterile water.

For killing the material Flemming's medium fluid was used. The material was imbedded in 52° paraffine and cut into sections eight and ten microns thick. The stains used were Flemming's triple, saffranin-gentian violet-orange G, and Pianeze. Both of these gave good results, but on account of the Pianeze being more convenient to handle it was used most.

Strips of the epidermis were removed from the leaf, placed on glass slides, and stained from one-half to one minute with orange G. The preparation was then rinsed with water and mounted in 50 per cent glycerine. The orange G stained the affected guard cells brown so they could be readily located and studied. By the use of the two methods the mode of entry of the organism and the subsequent development could be followed very closely.

Ritzema Bos (9) found that the fungus enters both through the stomata and cuticle of Narcissus. Mr. Ramsbottom (8) also found the fungus penetrating iris leaves by both methods. After making and examining many sections without finding a single case of direct penetration of the cuticle, the writer has concluded that such penetration must be of rare occurrence on iris.

Upon entering the substomatal chamber the germ tubes send out several branches which pass between the cells, wedging them apart. They occasionally penetrate the walls. The contents of the attacked cells coagulate and turn brown in the early stages, but seem to be used up later. The cell walls

turn brown, giving the characteristic color to the diseased spots. When the spots are about one-fourth inch in diameter stromatic masses are formed in the sub-stomatal chambers and give rise to tufts of conidiophores which push through the stomata. In growing through the stomata, the conidiophores tear the epidermis loose from the underlying tissues. This allows air to pass into the space thus formed and gives the characteristic gray color to the center of the spot (plate 6). In old lesions the mycelium becomes distinctly intracellular.

Vitality and longevity of conidia

The conidia remain viable for a comparatively short period of time, both in the field and in pure culture. As stated in connection with another heading, conidia taken from the field were found to be non-viable after November 26. The conidia were also found to disintegrate in pure cultures that were exposed to outdoor temperatures. It was at first thought that the disintegration was due to freezing, but after making many germination tests with spores from pure cultures of different ages, it was found that they lose their vitality and disintegrate at laboratory temperature after about 30 to 40 days, varying with the medium.

Mr. L. M. Massey of Cornell University stated in a letter to the writer that he had taken viable conidia from the field at intervals of two weeks throughout the winter of 1915-1916. These spores were in all probability produced during the winter months.

Overwintering of the fungus

In Wisconsin and other regions having similar weather conditions the fungus passes the winters as mycelium in the tissues of dead leaves and lesions on green leaves. Very little previous work has been done to determine the way in which the fungus, Heterosporium gracile, passes the winter. In studying this problem it was thought feasible to consider three possibilities, viz., (1) the overwintering of conidia, (2) the overwintering of mycelium in tissue of infected leaves, and (3) the overwintering in the perfect stage. Many infected leaves were examined up to December 20. No viable conidia were found after November 26, but the mycelium grew very readily when plated on culture media. The next examination of field material was made on March 14. No conidia were found at this time, but when the infected dead leaves were kept in a moist chamber in the laboratory for two days numerous viable conidia developed. Also, no subsequent examination of dead leaves showed the presence of conidia until April 26, as stated previously. When the snow had thawed out a few leaves, which remained green over winter, were found to have small brown spots on them. These spots were proved to be caused by Heterosporium gracile by isolating the fungus from some of them. Others were marked and left in the field. They produced conidia on April 26. This shows that the mycelium can live over winter both in the tissue of dead leaves and the lesions upon green leaves.

During the latter part of March and early part of April

structures were found developing in the tissues of dead infected leaves, which in the incipient stages gave promise of perithecia. Many sections of these structures were examined in various stages of development, but no asci or spores were ever found. During the early stages they were filled with oil globules. On April 26 these perithecioid stromata produced tufts of conidiophores and conidia (plate 7). Under certain conditions they may produce asci instead of conidia. And, although conidia are not produced throughout the winter in Wisconsin, the writer is of the opinion that they are produced at any time during the year in regions of milder climate.

V. CONTROL MEASURES

Thus far, very little work has been done toward controlling the leaf blotch of iris. Güssow (6) and Cooke (3) have recommended the removal of dead leaves. Mr. Ramsbottom (8) stated that the disease attacked only the lime loving species of Iris, and usually only when they were grown on soil deficient in this substance. He planned some experiments based on this theory, but for some reason they were never executed.

Ritzema Bos (7) showed that the disease of Narcissus induced by Heterosporium gracile could be practically controlled by the use of Bordeaux mixture.

After carefully following the development of the fungus during the late fall and early spring, it was decided to try two methods of control, and a combination of the two, viz.

(1) removal of the infected leaves, and (2) spraying with a solution of copper salts often enough to protect the plants from infection just after rains.

On March 30 all the dead leaves were removed from two small beds of German iris, and the old green leaves were cut off just above the surface of the ground. One of these beds was sprayed on May 3 with Bordeaux mixture 4-4-50, with soap used as a "sticker". The second was left unsprayed as a check. The leaves were not removed from two other beds, one of which was about a half mile from all others. Part of each bed was sprayed with Bordeaux mixture, 4-4-50 on May 3, the second part used as a check. The plots were sprayed the second time on May 19, this time with ammoniacal copper carbonate solution (5-3-50) with soap for a "sticker". This solution was used instead of Bordeaux to avoid staining the foliage, as the plants were coming into bloom. On account of the nature of the plants it was a difficult task to get the solution on all parts of the leaves.

So far no signs of the disease have developed in the beds from which the leaves were removed, either on the sprayed or unsprayed plants. A few lesions were found in one of the beds that was sprayed but did not have the leaves removed. Abundant infection developed on all the beds which were neither sprayed nor had the leaves removed.

These experiments have not been continued long enough or on a sufficient scale to warrant any definite conclusions,

but it seems that the results obtained up to date at least suggest a probable control. The writer hopes to follow out the plans during the summer.

SUMMARY

Iris leaf blotch is a parasitic disease of several varieties of "common" or German iris caused by Heterosporium gracile Sacc. The fungus has been reported as attacking Freesia, Antholyza, Hemerocallis, Gladiolus, Lychnis, Gemmengia chinensis, and varieties of Narcissus. On iris it manifests itself in elliptical spots with brown borders and grayish center. It sometimes kills the whole leaf, and in case of Narcissus, kills the whole plant.

European botanists have known the disease since 1881. It has been recorded from Britain, France, Italy, Germany, New Zealand, Holland, Greece, Denmark, Cape of Good Hope, Tyrol and Canada. In the United States it has been recorded from Ohio, New Jersey, Kansas, New York, and Wisconsin.

The disease is one of the most troublesome diseases of iris in England. It is also a very important disease of Narcissus in ~~Denmark~~ Holland. Not much is known of the economic importance of the disease in the United States.

The iris leaf blotch was first described by Saccardo (1881), who assigned the binomial Heterosporium echinulatum (B. & Br.) Cooke. He later changed the name to Heterosporium gracile (Wallr.). Wallroth's name was subsequently dropped, and the

binomial is now Heterosporium gracile Sacc.

The primarily important diagnostic characters of the fungus are the short, knotty, septate, olive-green conidiophores and the oblong-cylindric, 1-several septate and slightly constricted, echinulate, light olivaceous conidia, which are borne acrogenously and singly on small papillae. The description of Saccardo compares very favorably with the fungus studied by the writer, except that no muriform spores were ever observed.

The fungus was grown upon six media. The fungus grew slowly on all media and showed only minor differences on all substrata, except melilotus stems and cooked iris leaves. "Perithecioid", stromata were produced upon these, which resembled those produced in the dead tissues of infected leaves.

The perithecioid stromata found upon dead leaves produce conidia in late spring.

The minimal temperature for growth upon prune agar is below 4°C., the optimal about 22°C., and the maximal between 30° and 35°C.

Conidia grown in culture can withstand low temperatures for short periods of time.

The mycelium can live throughout the winter in pure culture. Overwintered cultures produce conidia during the spring.

Spores germinate readily in sterile water, prune decoction and agar media. They germinate in about two hours and thirty minutes at 22°C.

The minimal temperature for germination in water is below 4°C., the optimal, between 22° and 26°C., and the maximal about 30°C.

The fungus was isolated from the leaves by the spore dilution method and the "leaf fragment" method.

Three varieties of "common"iris, one of Siberica iris, the varieties of Narcissus Emperor and Pheasant, and one unknown variety of Hemerocallis were inoculated with single spore cultures of H. gracile. Mycelium and spores were used as a source of inoculum. Only the three varieties of common iris were infected. The period of incubation with spores is 5 to 8 days. When mycelium is used as the source of inoculum the induced lesions produce conidia within 6 to 8 days, while the lesions induced by spore infection produce conidia only after 35 days or more. The fungus was repeatedly reisolated and was found to possess the typical morphological characteristics of H. gracile.

Under favorable temperature conditions infection will take place if moisture remains on the leaf surface only 12 to 14 hours.

H. gracile is capable of inducing a dry rot of the root stocks of "common" iris.

Primary infection of iris leaf blotch appears 5 to 10 days after the first warm spring rain. In Wisconsin this occurs about the first week of May. Secondary infection takes place throughout the summer.

Spore production from primary infection begins about the first of June.

The sources of primary infection are conidia from dead infected leaves and conidia from lesions on green leaves which were infected the previous fall.

The organism enters through the stomata and becomes inter- and intra-cellular in the host tissue. The affected host cells die and turn brown. Stromata develop in the sub-stromatal chambers and produce conidiophores. In passing out through the stomata, the conidiophores tear the epidermis loose from the tissues underneath.

H. gracile overwinters as mycelium in the dead, and green leaves. Conidia may be produced throughout the winter in regions of mild temperature.

Control measures have been begun, but no conclusive results obtained. It seems probable that the destruction of all infected leaves in the fall or early spring will control it satisfactorily. It is difficult to reach all parts of large plants with spray solutions.

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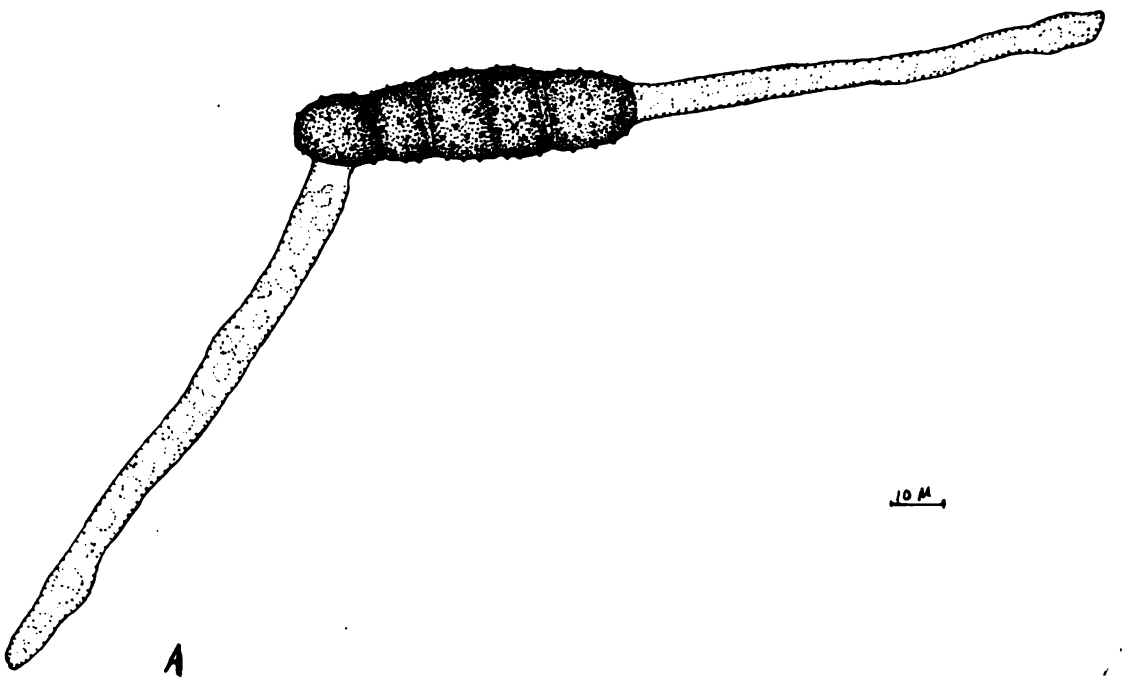
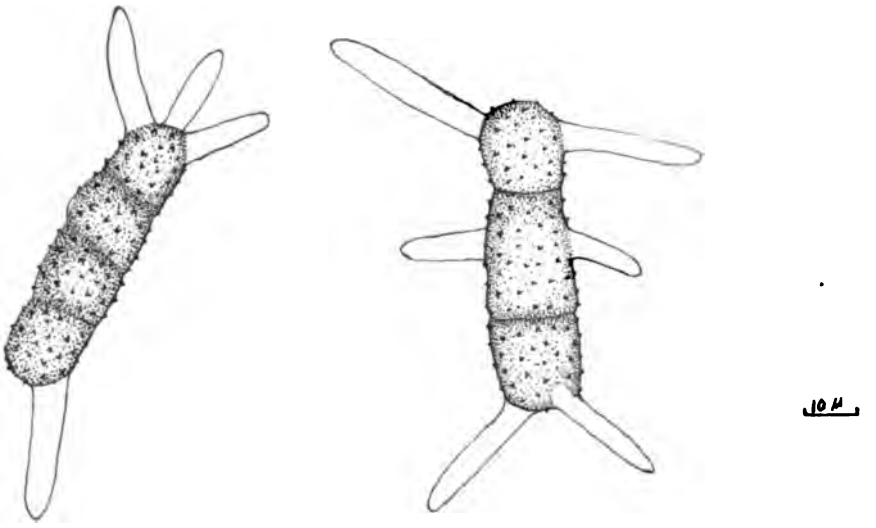


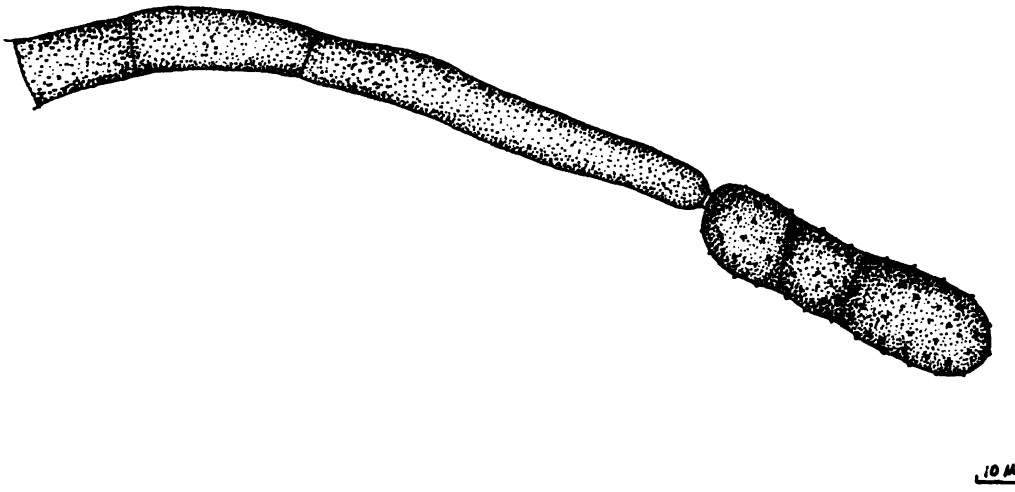
A



B

PLATE I

Camera lucida drawing of spores from melilotus stem culture.
A. After 48 hrs. at 30°C.
B. After 3 hrs. at 22°C.



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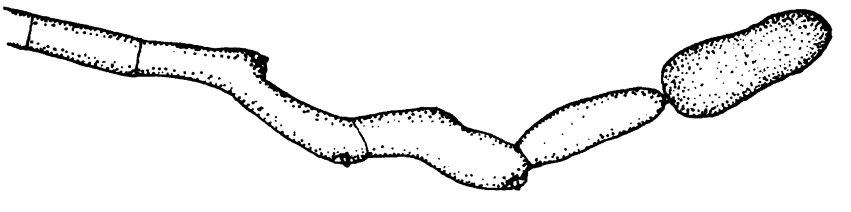

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PLATE II

Camera lucida drawings of conidiophores.

A. Conidiophore from perithecioid stroma.

B. Conidiophore from melilotus stem culture.

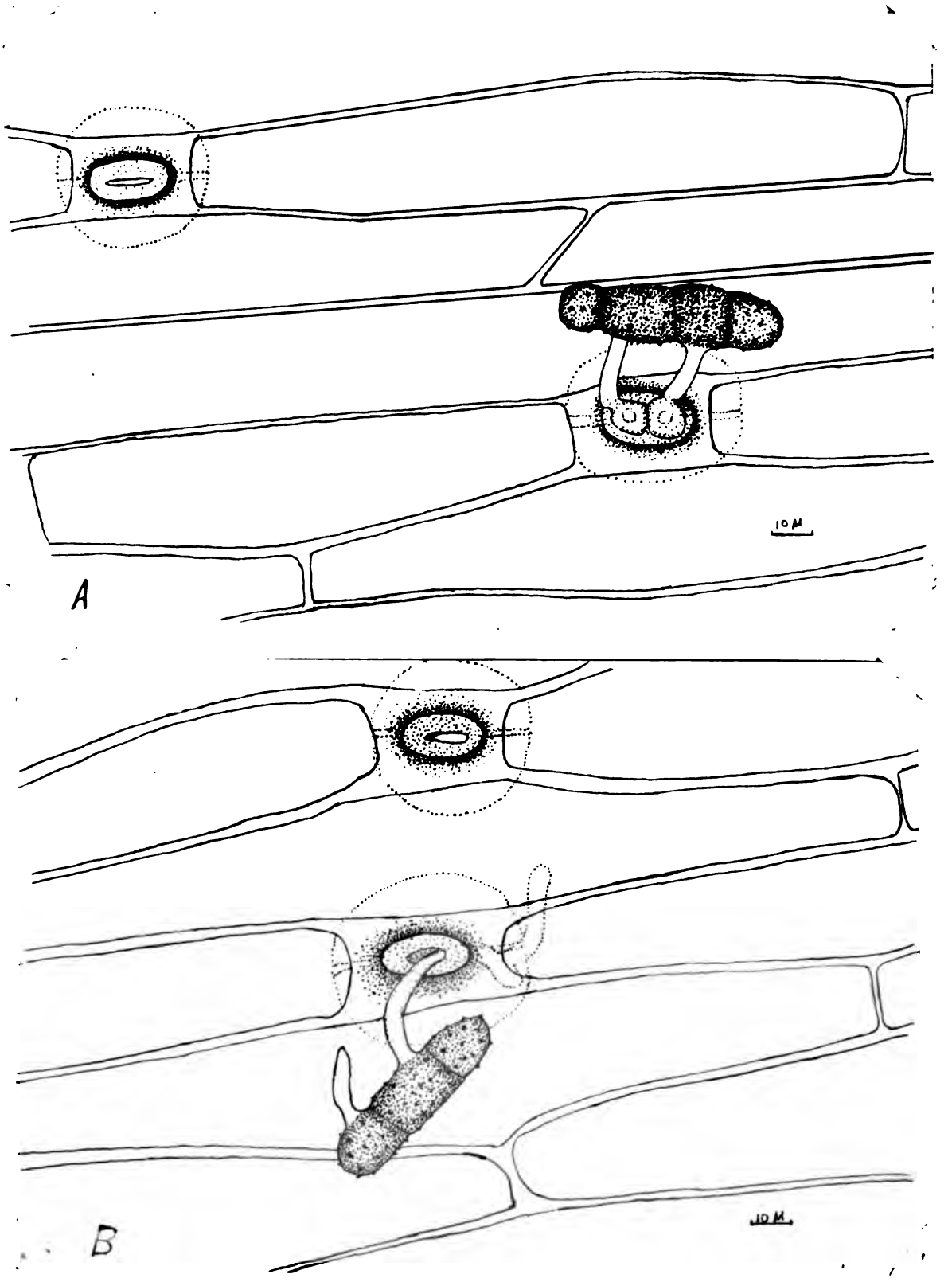
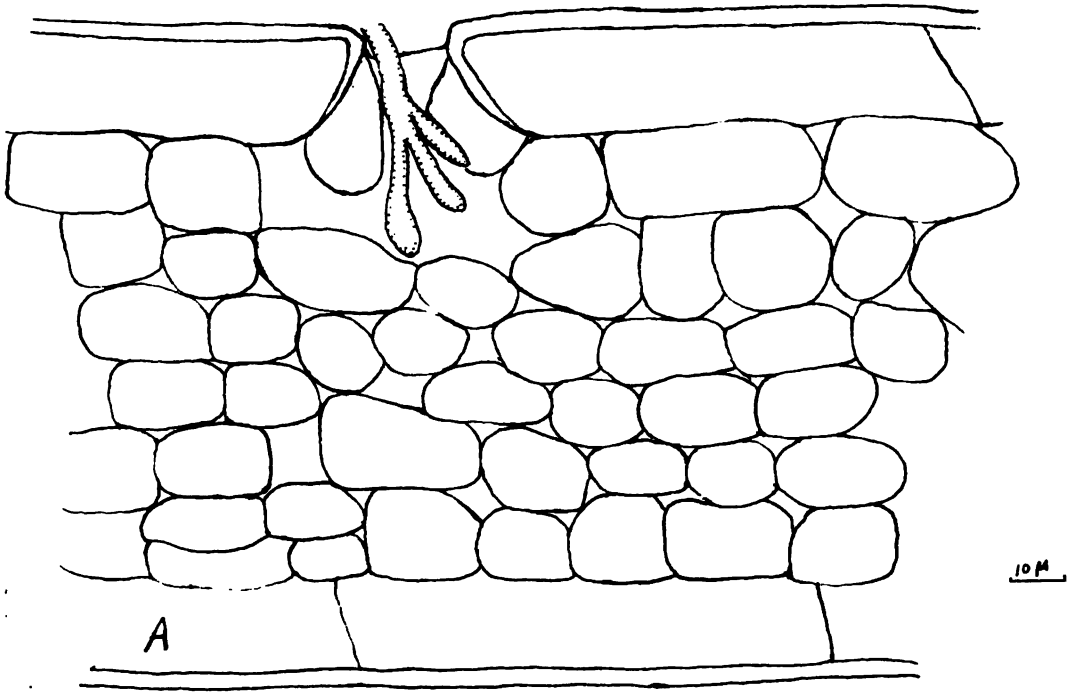


PLATE III

Camera lucida drawings of epidermis and spores.

A. Germ tubes entering stoma.

B. Germ tube extending into tissue beneath the epidermis.



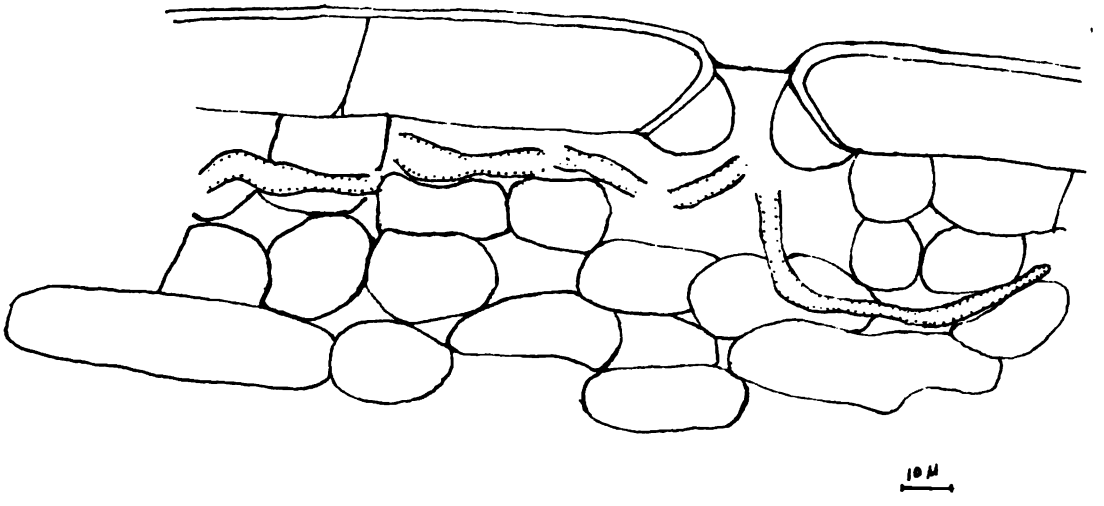


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PLATE IV

Camera lucida drawing of cross section of iris leaf.
A. Germ tube branching in substomatal chamber.
B. Hyphae developing in hypodermal tissues.

PLATE V

Photograph of artificially infected iris leaf 10 days after inoculation.

PLATE VI

Photograph of natural infection, showing gray center bearing tufts of conidiophores. Enlarged about 5 diameters.

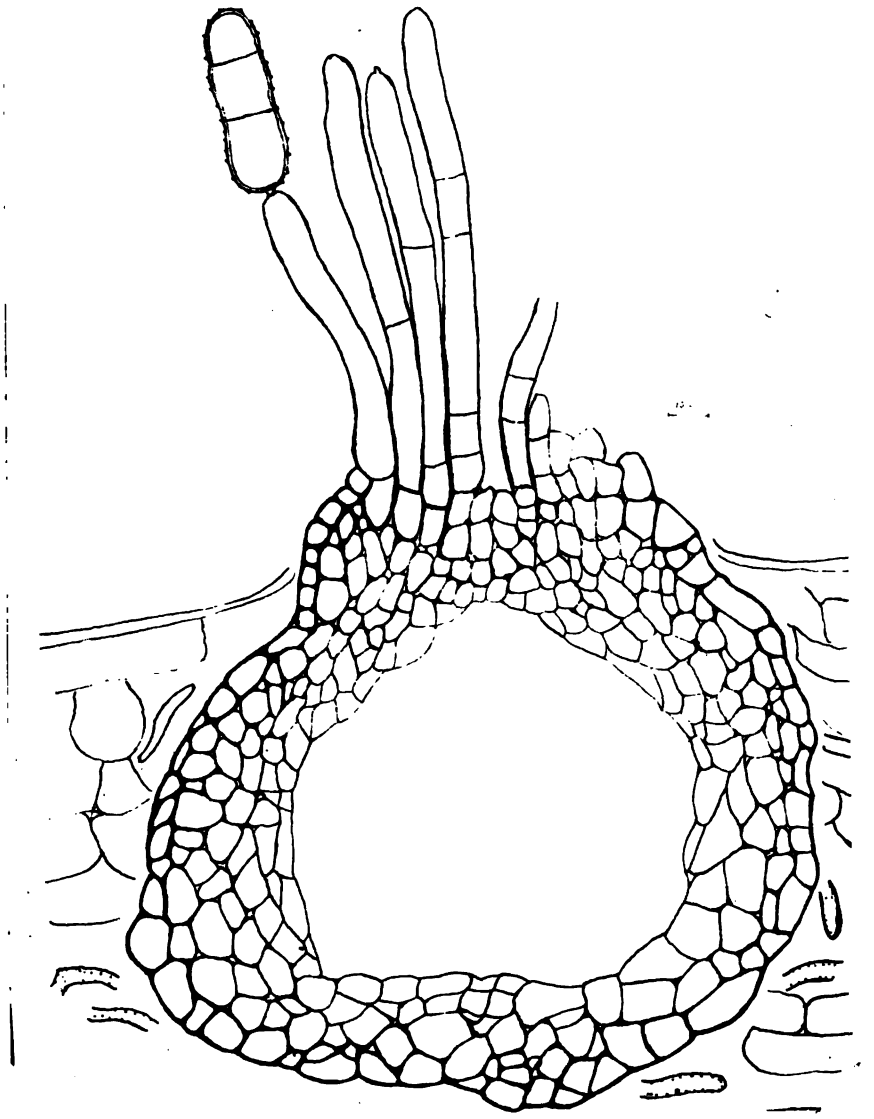

PLATE VII

Camera lucida of a median section of a perithecioid stroma in tissues of dead infected iris leaf.

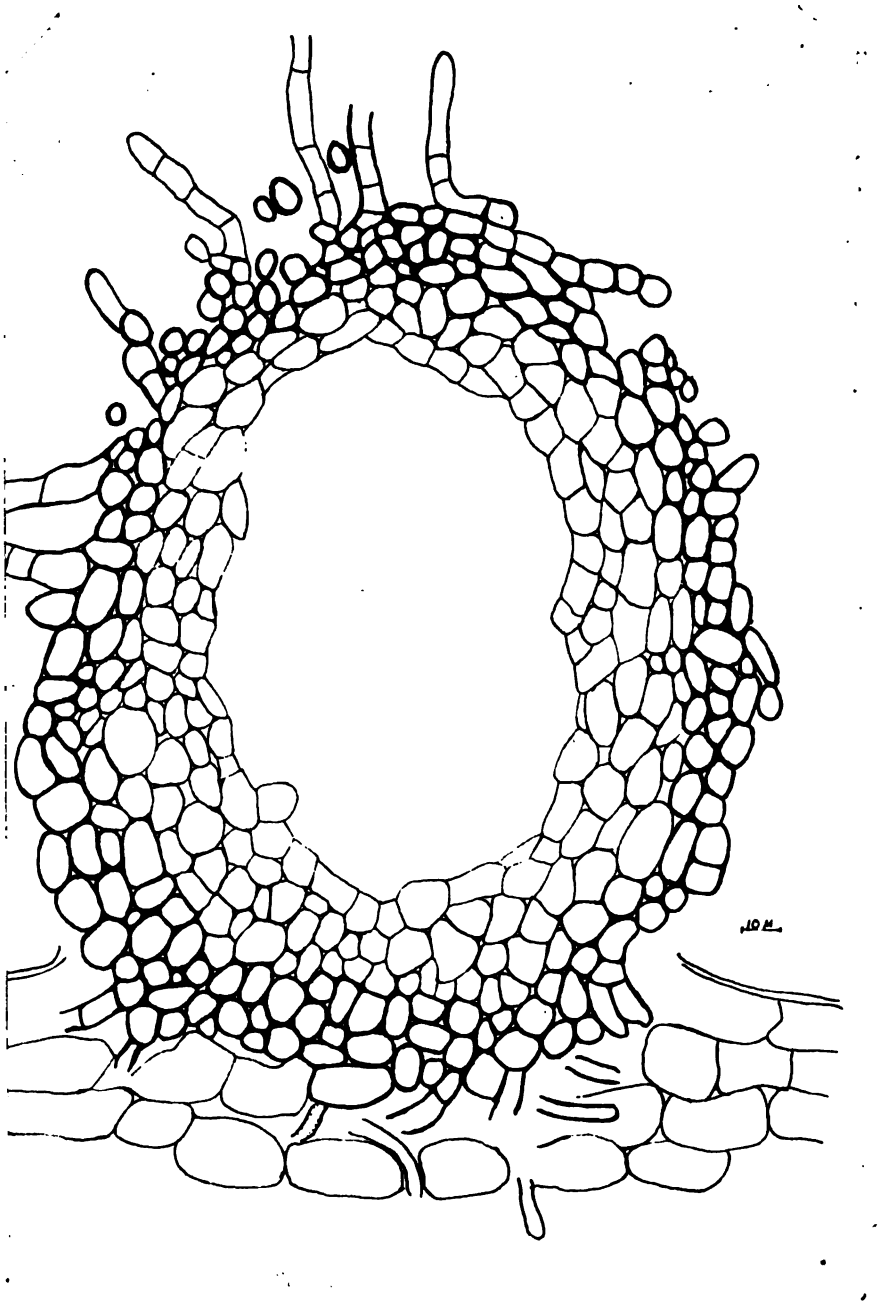

PLATE VIII

Camera lucida drawing of a median section of a perithecioid stroma produced on iris leaf in pure culture.

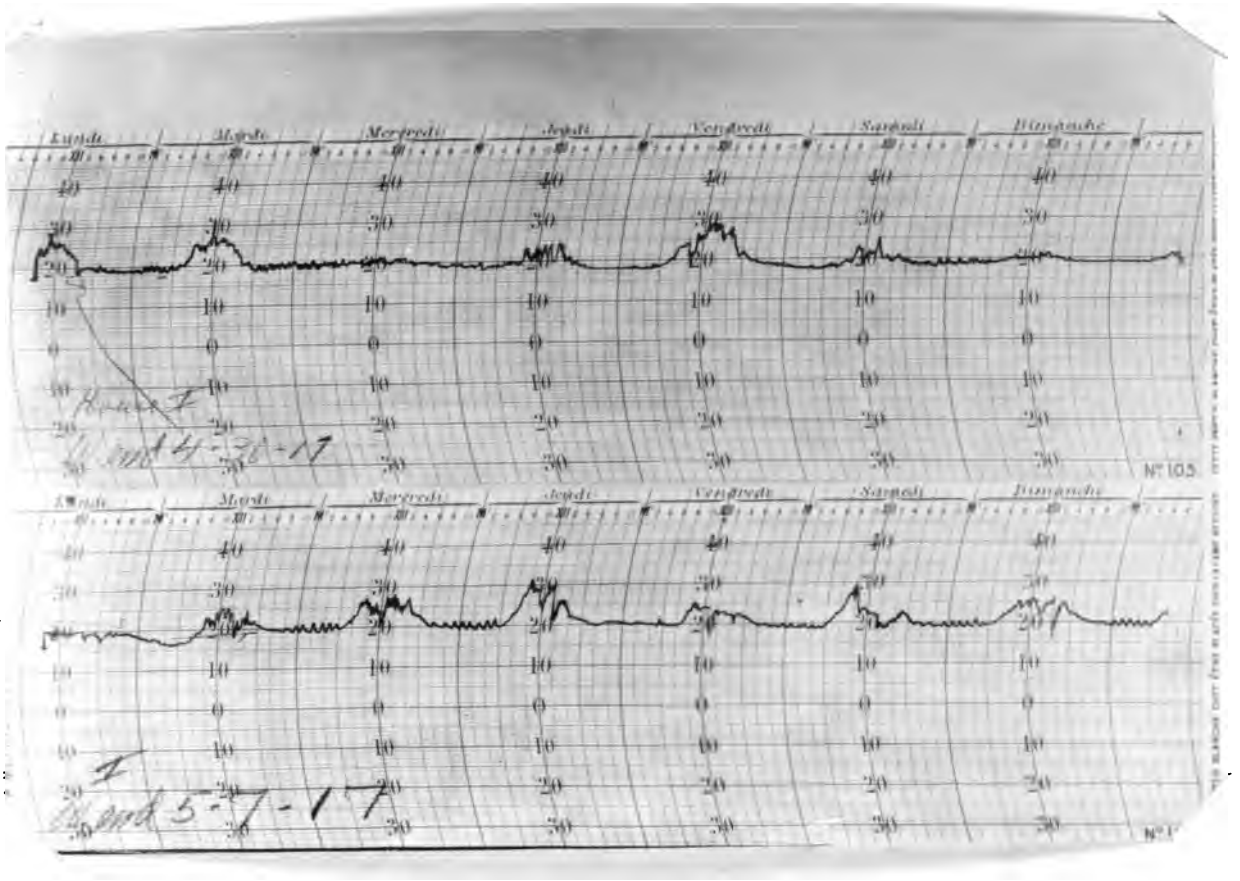

PLATE IX

Photograph of thermograph records showing range of temperature for two weeks at time one series of infection experiments was being conducted.

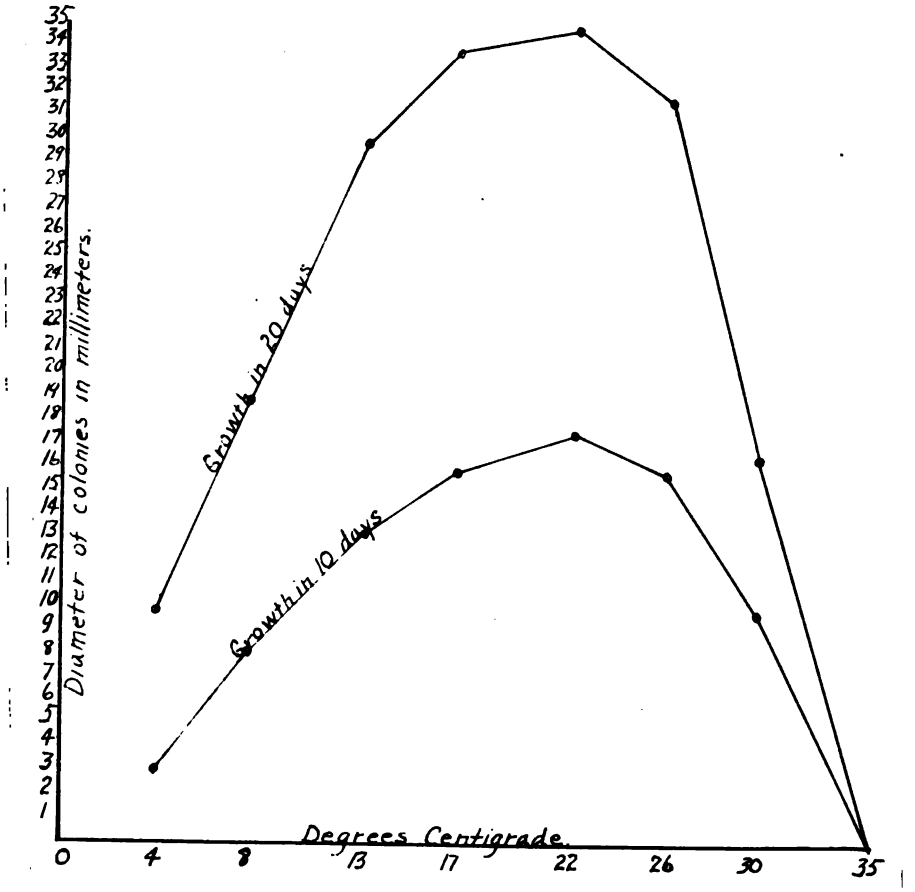

PLATE X

Curves showing relation of temperature to mycelial growth of H. gracile on prune agar.

PLATE XI

Photograph of iris leaves one week after inoculation with mycelium of H. gracile.

A. Control.

B. Not kept in moist chamber.

C. Kept in moist chamber one week.

Approved L. P. Jones

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